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5. (New) A method of determining the likelihood of metastasis of a prostate tumor in a human patient comprising assaying said tumor for FGF-R2 IIIc isoform mRNA, wherein expression of the FGF-R2 IIIc isoform mRNA indicates said tumor is likely to metastasize.

REMARKS

Reconsideration of this application and entry of the foregoing amendments are respectfully requested.

The specification has been amended to include sequence identifiers and to substitute the Sequence Listing submitted herewith on separate sheets for that submitted February 12, 2001. Entry of the attached Sequence Listing does not raise the issue of new matter as the sequence information contained therein is presented in the application as originally filed. The attached Sequence Listing corrects inadvertent errors that have been noted to be present in the sequences corresponding to sequence identifiers 41, 43 and 44 (as set forth in the February 12, 2001 Sequence Listing). The computer readable copy of the Sequence Listing submitted herewith is the same as the attached paper copy of that Listing.

The specification has been further amended to remove numerical citations noted by the Examiner and to correct certain spelling and punctuation errors.

Claim 1 has been revised as new claim 5 to define the invention with additional clarity. The new claim finds support throughout the disclosure, including in the claims as originally filed. Claims 2-4 have been cancelled merely to advance prosecution. Applicants reserve the right to pursue deleted subject matter in a continuation application.

Claims 1-4 stand rejected under 35 USC 112, second paragraph. Withdrawal of the rejection is in order for the reasons that follow.

The Examiner's objection to the phrase "metastatic potential" has been mooted by the above-noted revision of claim 1 as new claim 5. While the phrase does not appear in the revised claim, Applicants submit that it would be clear to one skilled in the art that the expression relates to the likelihood that the tumor will undergo metastatic spread outside the prostate (see page 2 of the application lines 5-8). Indeed, it is apparent from the Examiner's own comments that she had no difficulty appreciating the meaning of "metastatic potential".

The Examiner also objects to the expression "predominant expression". Again, while the phrase does not appear in new claim 5, it is submitted that one skilled in the art would have no difficulty with its meaning. This is evidenced by the fact that the phrase is used in the Carstens article (which appeared in a peer-reviewed journal) cited by the Examiner in rejecting the claims under 35 USC 102(a).

In view of the above, reconsideration is requested.

Claims 1-4 stand rejected under 35 USC 112, first paragraph. Withdrawal of the rejection is submitted to be in order in view of the above-noted claim revisions and for the reasons that follow.

The rejection appears largely based on the following allegations:

i) the specification does not teach the detection of the FGF-R2 IIIb and IIIc isoforms themselves or detection of the FGF-R2 IIIb and IIIc isoforms using antibodies, and

ii) activity of a cancer cell line is not necessarily reflective of the actual *in vivo* activity of that cancer type.

As regards the first point, the Examiner's attention is directed to the fact that the subject invention relates to FGF-R2 (fibroblast growth factor receptor 2) isoforms.

The invention is unconcerned with ferritin, ornithine, decarboxylase, p-glycoprotein or p53. These proteins are unrelated to FGF-R2. Thus, the relevance of the Examiner's comments regarding these unrelated proteins is not seen.

For a rejection to be properly based, it is incumbent on the examiner to provide a nexus between the teachings of the articles upon which he/she relies and the claimed invention. No such nexus is provided by the Examiner here.

As regards the second point, the Examiner is respectfully reminded that a patent applicant enjoys the presumption that his/her invention can be practiced as claimed. The Examiner bears the burden of providing evidence to the contrary. Here, the Examiner offers only sweeping assertions, wholly unsupported, to the effect that it is "well established in the art that conclusions drawn in cell lines cannot easily be extended to human patients". Such assertions do not constitute the required evidence.

The present invention is based on the finding that, in human prostate cancer, progression from an androgen sensitive tumor to an androgen insensitive tumor is accompanied by a change in alternative splicing of FGF-R2. This change results in a loss of the FGF-R2 (IIIb) isoform and predominant expression of the FGF-R2 (IIIc) isoform. As will be clear from the Carstens et al article cited

under 35 USC 102(a), using three well characterized human prostate cell lines and three metastatic prostate tumors, maintained as xenografts, it was shown that in both the 2 androgen independent cell lines and the androgen independent xenograft, androgen insensitivity was correlated with loss of the FGF-R2 (IIIb) isoform. Given this showing, no basis is seen for the Examiner's assertions regarding lack of correlation between *in vitro* and *in vivo* results.

Reconsideration is requested.

Claims 1-3 stand rejected under 35 USC 102(a) as anticipated by Carstens et al (1997). This publication is Applicants' own and a Declaration is submitted herewith that removes this publication as a reference. (For completeness, also submitted herewith is a Declaration that removes as a reference the article of Carstens et al (Mol. Cell. Biol. 18:2205 (1998) (copy attached)).

Reconsideration is requested.

Claims 1-3 stand rejected under 35 USC 102(b) as allegedly being anticipated by Yan et al. Withdrawal of the rejection is in order in view of the above-noted revision of claim 1.

Should the Examiner be inclined to make an obviousness rejection over Yan et al, she is urged to first consider

the attached articles that underscore the limitations of the rat model used by Yan et al in terms of mimicking the human condition. For example, Tennant et al (2000) (page 296 underlined) points out that the metastatic pattern observed with these rat tumors are different than the one observed in human cancer. In addition, there has been a debate about the true origin of the Dunning tumors as discussed by Bostwick et al (2000) (page 291 underlined) and by Goebel et al (1992) (abstract attached). Weerden and Romjin (2000) clearly indicate that laboratory animals (dogs excepted) do not develop prostate tumors upon aging (page 263 underlined) (this is also reported by Bostwick).

(The Examiner's attention is directed to the attached abstract by Applicants that describes alternative splicing of rat FGF-R2 in 2 rat prostate cell lines and that is believed to have appeared in August of 1997.)

In view of the above, reconsideration is requested.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached pages are captioned "**Version With Markings To Show Changes Made.**"

This application is submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

The paragraph beginning at page 11, line 8:

Figures 6A-6D. A critical 18 [nuclotide]
nucleotide sequence within the 57 nucleotide ISAR sequence
between Nde I and Nsi I nearly restores splicing regulation
in DT3 cells. (Fig. 6A) The 57 nucleotide ISAR sequence is
indicated at the top and deletions and mutants of this
sequence are shown as are control pBluescript sequences.
The 18 nucleotide "core sequence" (Rep1) is boxed, and
mutant sequences are underlined and in boldface. All
sequences were tested by deleting ISAR sequences from pI-
11-FS/Not/Cla-ISAR and inserting the indicated sequences
(SEQ ID NOS: 34-44). (Fig. 6B) SAR-20 and SAR 3' sequences
restore regulation, whereas SAR 5' does not. (Fig. 6C)
Mutations in the 18 nucleotide sequence shared by SAR-20
and SAR 3' (Mut 2 and Mut 3) cause loss of regulation
whereas a mutation outside this region (Mut 1) preserves
regulation. (Fig. 6D) One or three copies of the 18
nucleotide "core sequence" restore splicing regulation,
with three repeats of the sequence being slightly more

efficient than one repeat. Abbreviations are defined in descriptions of Figs. 2 and 3.

The paragraph beginning at page 12, line 11:

Figures 8A and 8B. Intron sequences important for regulation of rat and human FGF-R2 splicing display are highly similar. (Fig. 8A) Rat intron sequences corresponding to previously reported 21 nucleotide human sequence, IAS 2, which also mediates IIIb activation contain only one nucleotide difference (SEQ ID NOs: 45 and 46, respectively). (Fig. 8B) The 57 nucleotide rat ISAR sequence is highly similar to human sequences (SEQ ID NO: 47) in this same region, including the 18 nt shown to be most important for regulation (boxed sequences).

The paragraph beginning at page 13, line 10:

Figure 10. Rat FGF-R2 intron between exons IIIb and IIIc (SEQ ID NO:48).

The paragraph beginning at page 13, line 12:

Figure 11. Human FGF-R2 intron between exons IIIb and IIIc (SEQ ID NO:49).

The paragraph beginning at page 18, line 13:

Plasmid Construction. The pPIP11 adenoviral splicing construct is based on the L1 and L2 exons and is similar to the previously described pPIP7A [(36)], except that the sequence of the 3' exon between the Pst I sites and Hind III sites has been replaced by the sequence 5'-CTGCAGGACAACTCTTCGCGGTCTCTATGCATCCTCCGAACGGTAAGACCCTAAG CTT-3' (SEQ ID NO:1). The sequences of pPIP11 were PCR amplified with primers PIP10-F (5'-CCCGGGGGTACCGGGCGAATTCGAATT CGAGCTCACTC-3') (SEQ ID NO:2) and PIP11-R (5'-CCCGGGACTAGTAAGCTTAGGCT CTTGGCGTT-3') (SEQ ID NO:3). The PCR product was digested with EcoR1 and Spe I and inserted into the EcoR1 and Xba I sites of the eukaryotic expression vector PCDNA3 (Invitrogen). All cloning was done using standard methodologies. PCR amplification of genomic DNA from AT3 cells with FGF-R2 specific primers Int 3BF2 5'-CCGGACTAGTCACTACCGTTCTCCACCACT-3' (SEQ ID NO:4) and Int 3CR 5'-CCGGCTCGAGGGTCGGAAATCATTCGAAAC-3' (SEQ ID NO:5), and Intron 1F-5' CCGGACTAGTAAGCCCAAGGGGCCAGCAGT-3' (SEQ ID NO:6) and Intron 3R 5' CCGGCTCGAGACGAAGAG CCAAGGGCGCCT-3' (SEQ ID NO:7) yielded fragments FL and FS, respectively (Fig. 1B). These products were digested with Spe I and Xho

I and inserted into the Xba I and Xho sites of the pI-11 intron to yield pI-11-FL and pI-11-FS. Constructs derived from intron deletions in pI-11-FS as represented in Fig 4A (for example, pI-11-FS- Δ Bcl I/Nde I) were obtained by first cloning the FS sequences into the Spe I and Xho 1 sites of pBluescript (Stratagene), to generate pBlue-FS since these enzymes cut within PCDNA3 but not pBluescript. Deletions were performed by sequential digestion of pBlue-FS using the indicated restriction endonucleases. The digested ends were blunted using Pfu polymerase (Stratagene) and the resulting plasmids were gel purified, and religated using T4 DNA ligase. These plasmids were digested with Spe I and Xho I and the minigenes were cloned back into the Xba I and Xho sites of pI-11. Plasmids pI-11-IIIB-plus and pI-11-IIIB-minus were obtained using primers Int 3BF2 and Intron 2R2 5'-CCGGCTCGAGGGCTAGACATAGGAATGATT-3' (SEQ ID NO:8) and PCR amplification of pI-11-FS- Δ Bcl I/Nde I and pI-11-FS- Δ Bcl I/Nsi I, respectively. After Spe I and Xho I digestion the PCR products were cloned into the Xba I and Xho I sites of pI-11. Plasmids pI-11-IIIC-plus and pI-11-IIIC-minus were similarly obtained by PCR amplification using primers Intron 2F 5' CCGGACTAGTCAACGTTTTTGTGTTTGTGT-3' (SEQ ID NO:9) and Int 3CR to amplify pI-11-FS- Δ Bcl I/Nde

I and pI-11-FS- Δ Bcl I/Nsi I, respectively, followed by digestion with Spe I and Xho I, and insertion into the Xba I and Xho I sites of pI-11. pI-11-FS-Not/Cla-ISAR resulted from PCR of pI-11-FS using primers Nde/Not-F 5'-

CCGGCATATGGCGGCCGCC AAACAAATTCAAAGAGAAC-3' (SEQ ID NO:10)

and Nsi/Cla-R 5'-CCGGATGCATATCGATGCGATTGAA CACATGGAAAA-3'

(SEQ ID NO:11), digestion of these products with Nde I and

Nsi I and cloning into the Nde I and Nsi I sites in pBlue-

FS. The minigene sequence was removed with Spe I and Xho I

and cloned into the Xba I and Xho I sites of pI-11 to

generate pI-11-FS-Not/Cla-ISAR. The ISAR mutant constructs

pI-11-Not/Cla: Blue1, SAR 5', SAR 3', SAR-20, Mut 1, Mut 2,

Mut 3, Blue2, Rep1, and Rep 3 were obtained by deleting

ISAR from pI-11-FS-Not/Cla-ISAR using Not I and Cla I, and

then inserting annealed oligonucleotides with complementary

Not I and Cla I sites as represented by the following

oligonucleotide pairs: Blue1-F:

5'-GGAAGCGACTCCCCGTCGTGTAGATAACTACGATACGGGAGG

GCTTACCATCTGGCCCCAGTGAT-3' (SEQ ID NO:12); Blue1-R:

5'-CGATCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTA

TCTACACGACGGGGAGTCGC-3' (SEQ ID NO:13); SAR 5'-F:

5'-GGCCGCAAACAAATTCAAAGAGAACGGACTCTGTAT-3' (SEQ ID NO:14);

SAR 5'-R: 5'-CGATACAGAGTCCGTTCTCTTTGAATTTGTTTGGC-3' (SEQ ID

NO:15); SAR 3'-F: 5'-GGCCGCGGGCTGATTTTTCCATGTGTTCAATCGCAT-

3' (SEQ ID NO:16); SAR 3'-R: 5'-

CGATGCGATTGAACACATGGAAAAATCAGCCCGC-3' (SEQ ID NO:17); SAR-

20-F: 5'-GGCCGCCAAAGAGAACGGACTCTGTGGGCTGATTTTCCATGTAT-3'

(SEQ ID NO:18); SAR-20-R:

5'-CGATACATGGAAAAATCAGCCACAGAGTCCGTTCTCTTTGGC- 3' (SEQ ID NO:19); Mut1-F:

5'-GGCCGCCAAACTCTACGGACTCTGTGGGCTGATTTTCCATGTAT- 3' (SEQ ID NO:20); Mut1-R:

5'-CGATACATGGAAAAATCAGCCACAGAGTCCGTAGAGTTTGGC-3' (SEQ ID NO:21); Mut2-F:

5'-GGCCGCCAAAGAGAACGGACTCTGTGGGCTGAAAGATCCATGTAT 3' (SEQ ID NO:22); Mut2-R: 5'-

CGATACATGGATCTTTCAGCCACAGAGTCCGTTCTCTTTGGC-3' (SEQ ID NO:23); Mut3-F:

5'GGCCGCCAAAGAGAACGGACTCTGTGGGCTGATTTTTCACGCTAT- 3' (SEQ ID NO:24); Mut3-R: 5'-

CGATAGCGTGAAAAATCAGCCACAGAGTCCGTTCTCTTTGGC-3' (SEQ ID NO:25); Blue2-F:

5'-GGCCGCAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACACAT 3' (SEQ ID NO:26); Blue2-R: 5'-

CGATGTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTGC-3' (SEQ ID NO:27); Rep1-F: 5'-GGCCGCGGGCTGATTTTCCATGTAT (SEQ ID

NO:28); Rep1-R: 5'-CGATACATGGAAAAATCAGCCCGC (SEQ ID NO:29);

Rep3-F: 5'-GGCCGCGGGCTGATTTTCCATGTGGGCTGATTTTCCATGTGG

GCTGATTTTTCCATGTAT-3' (SEQ ID NO:30); Rep3-R:

5'-CGATACATGGAAAAATCAGCCCACATGGAAAA

TCAGCCCACATGGAAAAATCAGCCCGC-3' (SEQ ID NO:31). All plasmid

minigenes were prepared using plasmid maxi kits from

Qiagen. The identities all minigenes were confirmed by

automated DNA sequencing using an ABI sequencer.

The paragraph beginning at page 23, line 5:

RNA purification and RT-PCR analysis. Total cellular RNA was isolated from transfected cells using the method of Chomczynski and Sacchi [(12)]. 2 μ g of total RNA were heated to 100°C, chilled on ice, and reverse transcribed in a reaction volume of 20 μ l. containing 50 mM Tris-HCl (pH 8.3), 75mM KCl, 3mM MgCl₂, 10 mM DTT, 1mM dNTPs, 100 ng random hexamers, 2 U Rnasin (Promega), and 200 U MMLV-RT (Gibco) at 37°C for 1 hour. Samples were then heated to 90°C for 5 minutes, then chilled on ice. 2 μ l of each reverse transcription reaction was amplified in a 100 μ l PCR reaction containing 50 mM KCl, 10 mM Tris-HCl (pH 8.8), 1.5 mM MgCl₂, 0.1% gelatin, 200 μ M dATP, dGTP, and TTP, 50 μ M dCTP, 100 nM each primer, 10 μ Ci [α -³²P] dCTP, and 2.5 U Taq DNA polymerase (Boehringer Mannheim). Primers used were FGF-FB: 5'-CCCGGGTCTAGATTTATAGTGATGCCAGCCC-3' (SEQ ID NO:32) and FGF-RB: 5'-CCCGGGGAATTCACCACCATGCAGGCGATTAA-3'

(SEQ ID NO:33) for analysis of the endogenous gene and standard T7 and SP6 promoter primers for analysis of transfected minigenes. Amplification conditions consisted of an initial denaturation at 94°C for 4 minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 65°C for 30 seconds, and extension at 72°C for 1 minute, and a final extension at 72°C for 7 minutes. In all amplifications reactions, a water control and a mock reverse transcription control was included which resulted in no PCR product in all experiments. PCR products were directly added to restriction endonuclease digestions with either Ava I or Hinc II (New England Biolabs). Complete digestion using this protocol was always observed. Aliquots representing equal amounts of each PCR reaction with undigested and digested PCR products were loaded directly on 5% polyacrylamide non-denaturing gels at 100V for 3-4 hours, followed by drying and exposure to Amersham Hyperfilm-MP. Phosphorimager analysis was performed using a Molecular Dynamics Phosphorimager. Because equal amounts of Ava I and Hinc II digested PCR products were loaded onto each gel, quantification of exon IIIb or IIIc containing cDNAs (UBD or UCD) were obtained by using the quantification of the band at 380/377 bp which remained following Hinc II or Ava I digestion, respectively, as the

numerator. The denominator consisted of the sum of the bands remaining at 380/377 bp for each digest (UBD+UCD). When these results were also expressed with the contribution of IIIb and IIIc skipped products, the average value of the 232 bp band was also used in the sum of the denominator (UBD+UCD+UD), corrected for molar equivalents. Quantification of experiments using minigenes with only one (IIIb or IIIc) internal exon was determined as the sum of the 380 or 377 bp band divided by the sum of this same band and the 232bp band corrected for molar equivalents.

The paragraph beginning at page 25, line 2:

Nucleotide sequences of the introns flanking alternative exons IIIb and IIIc are highly conserved between rat and human. Because phylogenetic sequence comparisons often help to identify sequences with important functions, genomic DNA was sequenced from the regions of both rat and human FGF-R2 genes containing the alternative IIIb and IIIc exons, the constitutive exons located upstream and downstream of them, and the three introns between these exons. As seen in Fig. 1 the intron sizes flanking the alternative exons are roughly 1.1, 1.2 and 1.9 nucleotides in both rat and human genomic sequences. Henceforth, these exons will be referred to as intron 1,

intron 2, and intron 3, respectively. The University of Wisconsin Sequence Analysis Package GAP program was used to align the rat and human sequences for direct sequence comparison. As expected, the exon sequences were highly similar and corresponded to previously reported cDNA sequences for rat and human FGF-R2 [(25, 46, 65)]. Interestingly, the introns contained a number of regions with a very high level of sequence similarity and these regions were clustered around the IIIb and IIIc exons, whereas the intron sequences adjacent to the constitutive exons did not show an appreciable level of similarity. The rat sequence was screened and all intronic regions in which 90% of the nucleotides were identical to the corresponding human sequence for a stretch of at least 20 consecutive nucleotides were highlighted. These data are presented graphically in Fig. 1B. While some of these regions may represent evolutionary vestiges, it was also expected that regulatory sequences involved in mediating alternative splicing of these exons, which have been conserved from rat to human, would likely be represented by such conserved sequences. Thus, this information was used to direct the construction of a variety of minigenes, described below.

The paragraph beginning at page 26, line 9:

Minigenes pI-11-FL and pI-11-FS recapitulate the splicing pattern of the endogenous gene in AT3 and DT3 cells. An RT-PCR based assay was used that is similar to one used by other researchers investigating splicing of human FGF-R2 to assay for the splicing pattern of exons IIIb and IIIc [(14, 21)]. For analysis of the endogenous FGF-R2 transcript, RT-PCR was performed using primers (FGF-FB and FGF- RB) specific for the constitutive exons located upstream of IIIb and downstream of IIIc as shown in Fig.

2A. These products were separately digested with Ava I and Hinc II and analyzed by gel electrophoresis as demonstrated in Fig. 2B. Because exon IIIb contains an Ava I site not present in IIIc and exon IIIc contains two Hinc II sites not present in IIIb, IIIb inclusion is expected to result in a 367 bp product which is cut with Ava I but not Hinc II, and IIIc inclusion to result in a 364 bp product which is cut only by Hinc II. As expected, DT3 cells produce an FGF-R2 transcript which contains exclusively exon IIIb and AT3 cells consist entirely of IIIc containing transcripts; results which are consistent with the original report describing alternative splicing of FGF-R2 in these cells [(63)]. The validity of this assay was tested by

performing the same assay using titrations in which RNAs that contained only exon IIIb were mixed with RNAs containing only exon IIIc and it was observed that the proportion of each isoform seen by RT-PCR directly correlated with the fraction of the same isoform in the mixture. Thus, mRNAs containing exon IIIb or IIIc were amplified with equivalent efficiency in this assay. Furthermore, sequencing of these RT-PCR products verified that they were correctly identified by this approach.